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Qualitative ultrasound in acute critical illness muscle wasting

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Abstract

Rationale: A rapid and early loss of skeletal muscle mass underlies the physical disability common amongst survivors of critical illness. However, skeletal muscle function depends not only on its quantity but its quality, which may be adversely affected.

Objectives: To characterise the changes in *macroscopic* muscle echogenicity and fascial characteristics that occur early in critical illness, and to relate these to *microscopic* histologically-defined myofibre necrosis and fascial pathology.

Methods and Measurements: Thirty subjects comprised a subgroup of patients recruited to the Musculoskeletal Ultrasound in Critical Illness: Longitudinal Evaluation (MUSCLE) study. Comparisons were made between sequential Vastus Lateralis histological specimens and ultrasound assessment of Rectus Femoris echogenicity.

Main Results: Change in muscle echogenicity was greater in patients who developed muscle necrosis (n=15) than in those who did not (8.2%, (95%CI -5.3- 21.7), versus -15.0%, (95%CI -28.9- -1.09), p=0.016). The AUROC for ultrasound echogenicity's prediction of myofibre necrosis was 0.74 (95%CI 0.565-0.919, p=0.024) increasing to 0.85 (95%CI 0.703 -0.995, p=0.003) with the removal of those with potential iatrogenic muscle damage. Fasciitis was observed in 18 out of 30 biopsies (60%).

Conclusions: Myofibre necrosis and fascial inflammation can be detected noninvasively using ultrasound in the critically ill. Fasciitis precedes and frequently accompanies muscle necrosis. These findings may have functional implications for survivors of critical illness.

Background

Acute loss of skeletal muscle mass, greatest in patients with multi-organ failure, contributes to the ongoing physical disability common amongst survivors of critical illness (1, 2). However, skeletal muscle functional capacity depends not only on its quantity but its quality- recent data suggest that this, too, may be adversely affected in critical illness. It has been previously demonstrated that i) protein content (relative to that of Deoxyribonucleic Acid) decreases disproportionately more than radiologically-assessed muscle mass (2), ii) myofibre necrosis occurs in up to 40% of patients (2), and iii) the myosin/actin (contractile protein) ratio falls (3). Nonetheless, qualitative changes remain poorly explored and described, particularly at the whole muscle level.

Muscle ultrasound is increasingly used for assessment of muscle mass (4-6). Whole muscle and myofibre cross-sectional area changes are comparable in the critical care setting (2). Muscle ultrasound echogenicity (the differential acoustic impedance of tissue) (7) is normally low: healthy tissue contains little fibrous tissue, leading to little sound reflection (8). In disease, replacement of muscle with fat or fibrous tissue increases echogenicity (9). Increased echogenicity can discriminate between myopathies, neurogenic disorders and non-neuromuscular diseases in children (10, 11), and correlates with reduced strength and function with age (12-15).

Muscle echogenicity also increases in critical illness (16, 17) although its aetiology or associated histopathological characteristics remain unknown. Ultrasound can also identify fluid accumulation in fascial planes, to which inflammation and infection may substantially contribute (18, 19). Fluid shifts are commonly seen in critical illness, and whilst fascial pathology has not been described in the critically ill, ultrasound-detectable fasciitis is a well-described phenomenon in other disease states (20).

We thus sought to use B-mode ultrasound to characterise the changes in muscle echogenicity and fascial characteristics that occur early in critical illness, and to relate these to the presence of histologically defined myofibre necrosis and fascial histopathology.

Methods

Subjects comprised a subgroup of those patients recruited to the Musculoskeletal Ultrasound in Critical Illness: Longitudinal Evaluation (MUSCLE) study (NCT01106300,

www.clinicaltrials.gov) (2). In brief, appropriate ethics committee approval was obtained. Patients were recruited within 24 hours of admission to a university hospital and a community hospital (August 2009-April 2011). All were anticipated to (a) be invasively ventilated for >48 hours, (b) spend > 7 days in the intensive care unit (ICU) and (c) survive ICU. Patients were subsequently excluded if these criteria were not met. Patients were also excluded if pregnant, a lower limb amputee, or had a primary neuromuscular pathology or disseminated cancer. At enrolment, assent was obtained from the next-of-kin with retrospective patient consent obtained later.

Histological determination of myofibre necrosis, fascial pathology and muscle fibre type

Sequential Vastus Lateralis muscle biopsies acquired via the conchotome technique (21) (performed on days 1, 3, 7 and 10 of ICU admission) were examined for the development of muscle fibre necrosis (as previously reported (2)). Pre-existing muscle pathology was determined by screening for the presence of fibre atrophy (angular or polygonal fibres of <30μm diameter). Myopathic changes during admission were defined by the presence of one or more of the following: excessive fibre size variation and/or internal nucleation, whorled and split fibres, fibro-fatty infiltration, necrosis and regeneration, and any deviation in the fibre-typing expected at the biopsy site (checkerboard distribution of roughly one-third of type I, and two-thirds of type II fibres).

Quantification of type I and type II fibre numbers was performed on sections stained using Adenosine Tri-phosphatase enzyme (ATPase) histochemistry at pH 10.1 and anti-fast myosin heavy chain antibodies (Clone MY-32, www.sigmaaldrich.com).

Necrotic fibres were identified in Haematoxylin and Eosin (HE-) -stained sections as fibres with loss of cytoplasmic eosinophilia and basophilic nuclear staining, lytic changes and macrophage infiltration. Severity was assessed semi-quantitatively on a range from mild (single necrotic fibres) to severe (large groups or confluent anatomical zones of necrosis). Regenerating fibres were identified by their increased cytoplasmic basophilia, large vesicular nuclei and prominent nucleoli. Muscle fascia (deep fascia/epimysium, perimysial septa and endomysial connective tissue) was assessed for presence of oedema (interstitial fluid causing tissue pallor and separation between fascicles and individual fibres), fragmentation

and cellular infiltrates (neutrophil polymorphs, macrophages and lymphocytes). Sharply circumscribed groups of necrotic/regenerating fibres might have resulted from a previous biopsy, and affected samples were thus excluded from the analysis.

Muscle and fascial echogenicity

Ultrasound-assessment of Rectus Femoris (RF) anatomical cross sectional area (RF_{CSA}) in critical illness has been previously reported(2), as have methods of acquisition and data reliability (2, 22, 23). An excess of gel was placed on the skin to prevent pressure distortion, judged by maintenance of the convex shape of both the dermal layer and the superior RF border. These images were grey-scaled and RF echogenicity (RF_{ECHO}) determined by histogram analysis of pixel intensity (pixel distribution having been removed by grey-scaling images) of the pre-demarcated RF_{CSA} (Adobe Photoshop Elements 12, www.adobe.com). Images without standardized gain settings were not analyzed. The depth setting was chosen to visualize the femur on day 1, and the same setting was used on subsequent images. Three images were analysed for day 1 and day 10 timepoints, and the mean pixel intensity calculated. Fascial tissue echogenicity (FAS_{ECHO}) was determined from the same grey-scaled images using a standardised selection window applied to fascia overlying RF. Inter-image repeatability was assessed for all 30 subjects for two images taken on day 1, and the results available in the online supplement.

Statistical analysis

Data were collected prospectively. Data were assessed for normality using D'Agostino and Pearson omnibus normality tests, and analysed using paired two-tailed Student's t-test or Mann Whitney U test as appropriate. Normally-distributed data were described using mean (95% Confidence Interval) and non-normally distributed data as median (range). Categorical variables were analysed by $\chi 2$ testing. Multivariable and univariable linear regression analyses were applied (Statistical Package for the Social Sciences version 17 (SPSS, Inc, Chicago, Ill). Area Under Receiver Operator Curves (AUROC) was calculated for the ability of muscle ultrasound to predict our predefined primary endpoints of muscle necrosis and soft tissue infiltrate. Statistical significance was defined at the 95% level.

Results

Of the initial cohort of 37 patients with serial muscle biopsies and ultrasound, 30 patients had images acquired with standardised image settings. Characteristics of these patients are shown in Table 1.

Histological assessment of skeletal muscle biopsies

The proportion of Type I to Type II fibres did not change from day 1 to day 10 in either the cohort as a whole, nor differentially in those with or without chronic disease prior to critical illness (Table 2). Type I fibres lost 4.6% (95%CI -7.4—2.1) per day and Type II fibres 3.7% (95%CI -6.6—0.8) of cross sectional area per day, which was not significantly different (p=0.629).

Myonecrosis

As previously reported, myofibre necrosis was observed from day 7 in 15 of the 30 patients (2). Of these, four individual samples from different patients demonstrated sharply circumscribed necrotic areas, and were thus excluded from analysis (above). As previously reported, macrophages dominated the accompanying cellular inflammatory infiltrates(2). Our new analysis reveals that these macrophages are not merely infiltrating necrotic fibres, but also accumulate in the interfascicular septa and fascicular interstitium. Myopathic size variation, atrophy, myofibre necrosis, fascicular and fascial inflammation and oedema were all present in variable combinations.

Fascial Inflammation

Fascial pathology was observed in 18/30 biopsies. In at least 12 cases, the earliest changes were observed on day 1 or day 3, and comprised the accumulation of interstitial oedema with neutrophil polymorphs and fibrin largely within the deep fascia and perimysium, with very limited or absent myofibre necrosis. By day 7 or day 10, the fascial exudate was much more cellular with macrophages dominating. The infiltrate also extended into the fascicles

from the endomysium with accompanying necrosis and regeneration; generally the fascial pathology paralleled the severity of myofibre necrosis by days 7 or 10. In biopsies where the muscle-fascia interface was optimally represented, a pathological gradient was clearly discernible with more severe fibre damage in the fascicular periphery adjacent to the inflamed and oedematous fascia (Figure 1).

Relationships between parameters

Muscle echogenicity

RF_{ECHO} values were normally distributed. In a multivariate regression analysis, presence of chronic disease (drawn from the medical notes and hospital and general practice coding for management of chronic disease) was associated with RF_{ECHO} (r^2=0.22, p=0.028, Table 3). Patients with chronic disease (n=13) had higher baseline echogenicity than those without (n=17) (58.4 (95%CI 49.8-67) vs 44.1 (95%CI 36.1-52.1), p=0.013).

No relationship was seen between fluid balance and echogenicity (r^2=0.00, p0.899, n=30) or in the subgroups with and without necrosis (both r^2<0.01, p>0.5).

As a group, RF_{ECHO} did not change from admission over 10 days (-3.40 (95%CI -13.5-6.68)%, p=0.405). However, ΔRF_{ECHO} over 10 days was higher in those who developed muscle necrosis than in those who did not (8.2%, (95%CI -5.3- 21.7), n=15 versus -15.0%, (95%CI -28.9- -1.09), n =15, p=0.016), whilst baseline echogenicity did not differ between those with or without subsequent necrosis (42.66 (95%CI 33.6-51.8) vs. 48.4 (95%CI 41.2-55.6), p=0.296).

The AUROC for ΔRF_{ECHO} for prediction of myofibre necrosis was 0.74 (95%CI 0.565-0.919, p=0.024). The difference between groups was greater when those in whom iatrogenic muscle damage could not be ruled out were removed (17.8%, (95CI% 5.0-30.6), n=11 versus -15.0%, (95%CI -28.9- -1.09), n=14, p=0.001), with an increased AUROC of 0.85 (95%CI 0.703 -0.995, p=0.003) as seen in Figures 2 and 3 and the online supplement.

Fascial echogenicity

Admission FAS_{ECHO} was associated with admission RF_{ECHO} (r^2=0.237, p=0.006) but not with other potential variables (Table 4).

In the cohort overall, FAS_{ECHO} did not change over 10 days (2.0(95%CI -7.32-11.3)% n=30, p=0.598). Amongst individuals, change in FAS_{ECHO} (ΔFAS_{ECHO}) was correlated with ΔRF_{ECHO} (r^2=0.22 (95%CI 0.116-0.750) p=0.009). The correlation remained significant with the exclusion of those patients in whom iatrogenic damage could not be ruled out (r^2=0.20 (95%CI 0.060-0.763), p=0.024 figure 4).

Discussion

The presence of histologically-confirmed fasciitis (found in the majority of the critically-ill patients studied) correlated with that of ultrasound-identified fascial oedema. Oedema, neutrophil infiltration and fibrin deposition dominated the early phase of critical illness, with macrophage-rich cellular fasciitis extending deeper within the muscle fascicles. Accompanying myofibre necrosis and muscle regeneration appeared later. The presence of myofibre necrosis was predicted by increased ultrasound echogenicity during the first week of critical illness. There was no change in the proportion of type 1 and type 2 myofibres observed during the first week of critical illness.

Patho-radiological relationship in the first week of critical illness

B-mode ultrasound determination of muscle cross-sectional area as an index of muscle mass(2, 5, 22, 23), correlates well with both athletic performance in healthy subjects (24, 25) and with skeletal muscle function in chronic disease (22, 23). Age-related changes in muscle quality are associated with reduced function (13, 14), and may be the result of infiltration with fat or collagen (26). In addition, qualitative changes have been reported in some chronic disease states and have been related to impaired physical function(13, 14, 26).

B-mode ultrasound was able to detect the development of skeletal muscle necrosis. Such muscle necrosis appears anatomically patchy (2, 27-29), which may account for the few patients with histologically-diagnosed necrosis who lacked a corresponding change in

muscle echogenicity: the ultrasound window may not have captured the presence of necrotic fibres.

Critical illness myopathy has been classified by the presence of 1) non-specific alterations, 2) selective loss of thick filaments, and, 3) acute necrotisis (30). The presence of fascial pathology in critical illness has, to our knowledge, not been characterised before. Histologically-diagnosed fasciitis was present in a majority of our patients and associated with increased ultrasound echogenicity, in keeping with similar findings in cases of necrotising (18, 19, 31, 32), plantar(33-35), eosinophilic (36, 37) and non-eosinophilic fasciitis (38). Furthermore we clearly identified sequential changes with oedema, neutrophil polymorphs and fibrin dominating the early phase (days 1-3), and a macrophage-predominant cellular fasciitis featuring in the late phase (days 7-10), extending deeper within fascicles with accompanying myonecrosis and regeneration. The association with muscle necrosis implies either a common causal link, or an anatomically related "spill-over"; as yet no pathophysiological process has been associated with the development of myonecrosis. Through its association with pain (39), loss of range of movement and reduced flexibility (40), fasciitis could have important associated clinical sequelae and adversely effect early mobilisation and rehabilitation, two crucial interventions in preventing and treating Intensive care acquired weakness (41). Furthermore, persistence of macrophages in skeletal muscle and temporal plasticity in phenotypes (M1 to M2) may adversely affect satellite cell function and regeneration, and potentiate fibrosis.

No change in myofibre proportion was seen between biopsies over the 10 days of study. A shift in myofibre phenotype towards an increased proportion of fast- contracting type II fibres, and type IIb fibre-specific atrophy is a consequence of disuse, steroid treatment and related to a number of clinical conditions including chronic respiratory ambulant disease (42). Given the narrow timecourse of the present study, it is not particularly surprising that we did not observe any changes in the major fibre types, although we did not distinguish between the relative proportions of the two fast fibre subtypes and did not analyse type-specific fibre atrophy. That later biopsy may have revealed some evidence of fibre shift and/or type-specific atrophy, cannot be excluded.

An overall positive fluid balance is not unusual in critical illness. However, independently of such changes, alterations in factors such as oncotic pressure and microvascular permeability may alter fluid distribution between the vascular and tissue compartments. Whilst no relationship was seen between fluid balance and change in echogenicity, fluid balance thus remains a crude surrogate for fluid state, and does not distinguish between intravascular, intracellular and third spaces and fluid shifts between them.

Study limitations

Several limitations of the study have been discussed, and sample size remains an issue, especially in the context of a radio-pathological validation study. Nevertheless these data represent the largest serial muscle biopsy and radiological assessment to date- performing larger scale studies may neither be feasible nor ethically appropriate. Comparing biopsies of Vastus Lateralis with ultrasound measurements of Rectus Femoris may lead to potential bias. This is an unavoidable methodological issue in unstable ventilated patients, as Vastus Lateralis cross sectional area measurements requires patient repositioning (best avoided on non-clinical grounds), and Rectus Femoris is anatomically related to large vascular structures (making biopsy hazardous). . Whilst these muscles are structurally and functionally different components of Quadriceps Femoris, previous reports have validated RF_{CSA} as a measure of change in muscle mass (2). Had ultrasound and biopsies been performed on the same muscle, the predicative capacity of ultrasound may have been greatly enhanced. A benefit of this method is that iatrogenic contamination of the ultrasound measurements is unlikely to be an issue.

Future studies might broaden subject recruitment, and extend the period of study beyond the first ten days providing greater insight into muscle wasting and changes in architecture during the recovery phase of critical illness. The lack of premorbid functional data limits our ability to infer the effect of changes in muscle echogenicity (and therefore muscle necrosis) may have on functional impairment.

Clinical implications

Whilst what constitutes a 'clinically significant' change in muscle echogenicity remains unknown, muscle echo intensity is negatively correlated with muscle strength (12, 14, 43) and performance (13), independent of muscle mass. The association between change in muscle echogenicity and the presence of muscle necrosis may explain the significant variation in change in muscle echogenicity seen in published ultrasound data (without contemporaneous histological examination) in critically ill patients (17).

Inflammation of skeletal muscle fascia is common amongst the critically ill, and precedes the development of myonecrosis. Both can be detected by ultrasound- a simple, inexpensive and widely available tool that may facilitate the investigation of the relationship between muscle necrosis and fasciitis, impaired functional outcome, and the genesis of the generalised body pain so common in critical care survivors (1). Detailed characterisation of the immune inflammatory response may shed light on the yet poorly understood mechanisms that could causally link fascial and muscle inflammation in during acute critical illness. Future outcome studies, and those focused on rehabilitation, may be able to use admission muscle mass and muscle quality to identify those likely to respond less well to resistance training and rehabilitation (43).

Future research should focus on both the aetiology of muscle necrosis, and its effect on functional capacity in both the acute phase and on long term disability. The presence of muscle necrosis may be responsible for failure to respond to early rehabilitation (44). Single skeletal muscle fibre assessments of muscle contraction (45), the interplay between myonecrosis, systemic inflammation (46) and regeneration (47) need to be examined. Comparative work with other non-invasive methods of assessment of muscle bulk and quality should be performed (48). Importantly, muscle echogenicity may be able to assess novel interventions (49) for potential muscle damage (50).

Conclusions

Myofibre necrosis and fascial inflammation in critically ill patients can be detected noninvasively using B-mode ultrasound. Fasciitis precedes and frequently accompanies muscle necrosis, and is dominated by macrophages in the sub-acute phase. The effect of

these qualitative changes in skeletal muscle on early mobilisation, rehabilitation and subsequent functional debility warrant further investigation, in tandem with the pathophysiology underlying fascial and muscle inflammation and necrosis.

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Figure Legends:

Figure 1: Fascial and Myofibre pathology in skeletal muscle biopsies from critically ill patients.A, B: Sections from a control muscle biopsy showing histologically normal appearances. The deep fascia condenses on the surface of the fascicles forming the epimysium (Star, A). Perimysial connective tissue septa branch off from the epimysium dividing the muscle in to smaller fascicles (Star, B). The endomysium comprises the extracellular matrix surrounding individual myofibres. C: Section from patient taken on day 1 shows pre-existing myopathic changes comprising mild variation in fibre size and increased internal nuclei. D: Day 3 biopsy taken showing prominent oedema with a few neutrophil polymorphs and some fibrin in the epimysium. The adjacent fascicle is largely unaffected. E, F: Day 10 biopsy from the same patient shows a cellular inflammatory infiltrate in the fascia extending deep in to the adjacent fascicles with accompanying myonecrosis and regeneration. High magnification image from the same region (F) shows the inflammatory cells to be predominantly macrophages. G: Day 3 biopsy showing mild oedema and a few inflammatory cells in the perimysium (Star). The adjacent fascicles show rare necrotic fibres, but appear largely intact. H: Day 10 biopsy from the same patient (G) that shows a more florid, cellular, macrophage-predominant destructive fasciitis with myonecrosis. I: Day 7 biopsy showing epimysial oedema and inflammation. A 3-4 cell thick sliver of the adjacent fascicular periphery shows marked pathology with myonecrosis and regeneration (inset) and a decreasing gradient of abnormality towards the fascicular centre. All biopsies were taken from the vastus lateralis and pathology was assessed on frozen sections stained with haematoxylin and eosin.

Figure 2: Percentage change in Rectus Femoris echogenicity ($\%\Delta RF_{ECHO}$) over 10 days for those patients that developed myofibre necrosis and those that did not. Those patients in who Iatrogenic necrosis could not be ruled out are marked with a clear box

Figure 3: Representative paired ultrasound and haematoxylin and eosin stained sections of a patient on day 1 and day 10, demonstrating the reduction in Rectus Femoris Cross Sectional Area, an increase in Rectus Femoris echogenicity and the presence of myofibre necrosis with

cellular infiltrate on day 10. Abbreviations: F=Fascial layer, RF=Rectus Femoris, VI=Vastus Intermedius and B=Femoral Bone

Figure 4: Relationship between muscle and fascial echogenicity over 10 days, n=30. Those patients in who Iatrogenic necrosis could not be ruled out are marked with as open circles. $\%\Delta FAS_{ECHO}$ = Percentage change in fascial echogenicity in 10 days. $\%\Delta RF_{ECHO}$ =Percentage change in Rectus Femoris echogenicity over 10 days